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Evidence for different, host-dependent functioning of *Rx* against both wild-type and recombinant *Pepino mosaic virus*.

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The potato *Rx* gene provides resistance against *Pepino mosaic virus* (PepMV) in tomato, however recent work suggests that the resistance conferred may not be durable. Resistance-breaking can likely be attributed to multiple mutations observed accumulating in the CP region of resistance-breaking isolates, but this has not been confirmed through directed manipulation of an infectious PepMV clone. The present work describes the introduction of two specific mutations, A-T78 and A-T114, into the coat protein minimal elicitor region of an *Rx*-controlled PepMV isolate of the EU genotype. ELISA analysis and phenotypic evaluation was conducted in three *Rx*-expressing and wild-type solanaceous hosts; *Nicotiana benthamiana*, *Nicotiana tabacum* and *Solanum lycopersicum*. Mutation A-T78 alone was sufficient to confer *Rx*-breaking activity in *N. benthamiana* and *S. lycopersicum*, while mutation A-T114 was found to be associated with a secondary, likely compensatory, A-D100 mutation to break *Rx*-mediated resistance in *S. lycopersicum*. These results suggest that the need for a second, fitness-restoring mutation, may be dependent on the PepMV mutant under consideration. Both mutations conferred *Rx*-breaking in *S. lycopersicum*, while neither conferred *Rx*-breaking in *N. tabacum* and only A-T78 allowed *Rx*-breaking in *N. benthamiana*, suggesting that *Rx* may function differently depending on the genetic background in which it is present.

The plant immune system is multilayered, consisting of both broad spectrum and specific lines of defence. Dominant resistance (*R*) genes constitute an important component of these specific defence mechanisms. The products of *R* genes recognise pathogen avirulence (*Avr*) molecules and trigger a highly effective resistance response in a race-specific manner. Recognition and resistance depend on factors expressed from both the pathogen and the host, and are therefore described as a gene-for-gene interaction system (Flor, 1971). The triggered resistance commonly involves the induction of the hypersensitive response (HR) (Hammond-Kosack and Jones, 1996), a form of programmed cell death resulting in necrosis at the site of infection, thereby preventing systemic viral spread.

The *Rx* gene from potato provides resistance to *Potato virus X* in commercial potato accessions (Cockerham, 1970). It encodes a NBS-LRR type protein with a coil-coil (CC) domain at the N-terminus (CC-NBS-LRR) (Bendahmane *et al*., 1999). The C-terminus of the LRR domain is thought to be involved in specific recognition of the pathogen elicitor (Dangl and Jones, 2001; Farnham and Baulcombe, 2006). Co-expression studies have demonstrated intramolecular interactions between the CC-NBS and LRR domains to be integral in the functioning of the Rx protein. Presence of the pathogen elicitor disrupts these interactions leading to Rx activation and defence signaling initiation (Moffett *et al*., 2002).

The resistance conferred by *Rx* is unusual in that it does not involve an HR. Viral replication has been reported to be halted in the initially infected cell and cannot therefore be detected at tissue level. For these reasons, the term ‘extreme resistance’ (ER) has been coined to describe it (Bendahmane *et al*., 1999; Tozzini *et al*., 1991). The PVX

capsid protein (CP) is the sole elicitor of the *Rx*-based resistance response (Bendahmane *et al*., 1995, 1999; Goulden *et al*., 1993). The resistance conferred is described as durable since only a single resistance-breaking isolate is known, PVX_{HB} (Jones, 1985; Moreira *et al*., 1980). Mutational analysis has shown that the mutation of a conserved CP residue was sufficient to overcome *Rx*-mediated resistance (Goulden *et al*., 1993). However, PVX isolates carrying this mutation are severely affected in their ability to mount a systemic infection in potato and a second, fitness-restoring mutation in the CP is necessary for full infectivity in resistant potato varieties. This need for two mutations to gain a full resistance-breaking phenotype probably accounts for the durability of *Rx* resistance (Goulden *et al*., 1993).

A number of *R* genes have been shown to retain their effectiveness when transgenically introduced into heterologous plant species (Baurès *et al*., 2008; Rommens *et al*., 1995; Song *et al*., 2003; Spassova *et al*., 2001; Whitham *et al*., 1996). *Rx* has been shown to be active against a range of potexviruses in transgenic *Nicotiana* spp., even when as little as 40% homology exists between the CPs of the viruses concerned (Baurès *et al*., 2008; Candresse *et al*., 2010). Due to this unusually broad activity range, it has been proposed that *Rx*-based recognition is dependent on conserved structural elements of the viral CP rather than on a linear amino acid sequence (Baurès *et al*., 2008; Chapman *et al*., 1992; Goulden and Baulcombe, 1993). Transient expression of CP fragments from PVX, *White clover mosaic virus* (WClMV) and *Narcissus mosaic virus* (NMV) has allowed the identification of a 90 aa ‘‘minimal elicitor’’ region required for *Rx*-based recognition (Baurès *et al*., 2008).

Pepino mosaic virus (PepMV), an emergent potexvirus that presents a major threat to tomato production, also possesses the ability to infect a number of other solanaceous crops. Despite control efforts, tomato-infecting isolates of PepMV have gained a worldwide distribution in just over 10 years (Hanssen *et al*., 2010). A high level of conservation is displayed between all sequenced PepMV isolates in the *Rx* minimal elicitor region of the CP (Candresse *et al*., 2010). *Rx* has been shown to be active against PepMV providing initial hopes that it may provide a valuable source of resistance in susceptible crop species. However, recent evidence indicates that *Rx*-based resistance against PepMV in tomato may not be durable. Candresse *et al.,* (2010), passaged PepMV through *Rx*-expressing tomato and reported the frequent selection of resistance-breaking isolates. Sequence analysis of the CP of these variants showed the accumulation of a number of point mutations in the *Rx* minimal elicitor region that were proposed to be affecting *Rx*-mediated recognition (Candresse *et al*., 2010). However, the precise impact of the observed mutations could not be confirmed in the absence of an infectious clone (IC) in which to introduce the suspected point mutations.

Using our recently described infectious clones of an EU genotype of PepMV (Duff-Farrier *et al*., 2014; GenBank accession; KJ018164), we report the analysis of the impact of two of these candidate point mutations (A-T78, A-T114) in the *Rx* minimal elicitor region. The *Rx*-breaking activity of the resulting PepMV mutants was investigated in three different transgenic *Rx*-expressing hosts; *Nicotiana benthamiana*, *N. tabacum and Solanum lycopersicum*.

The desired mutations (A-T78 and A-T114) were introduced into the CP region of a wild-type PepMV EU IC, constructed as described previously and contained within a pYES2

vector; pYES2_EU (Duff-Farrier *et al*., 2014). pYES2_EU was used as the template in Phusion PCR (Thermo Scientific, Wilmington, DE, USA) to amplify the PepMV CP region (Primers 1+2, Table 1), which was cloned into pJET1.2 (Thermo Scientific), forming pJET1.2_CP. This was entered into two site-directed mutagenesis reactions using a GeneArt® Site-Directed Mutagenesis System (Invitrogen), according to the manufacture's protocol; A-T78 and A-T114, primer sets 3+4 and 5+6 respectively (Table 1). The mutagenised CP regions were excised and entered into a yeast recombination reaction with digested pYES2_EU and a linearised pYES2 backbone, following the protocol from Gietz *et al*., (2002). Restriction digestion and sequencing confirmed successful construct generation for both pYES2_EU_A-T78 and pYES2_EU_A-T114.

A Riboprobe® SP6 System (Promega, Madison, WI, U.S.A.), in conjunction with a Ribo m7G Cap Analog (Promega) was used to generate infectious transcripts *in vitro* from wild-type, A-T78 and A-T114 *Kpn*I linearised templates (Foster and Turner, 1998; Turner *et al*., 1994, 1999). Each reaction was immediately inoculated onto the surface of two *N. benthamiana* plants at the three-leaf stage. Plants were kept under greenhouse conditions (18°C with a 16h/8h: light/dark cycle). ELISA analysis at 21 dpi showed high absorbance values for all constructs, indicating successful establishment of infection in all instances. All ICs displayed systemic phenotypes akin to that of the mild EU IC, characterised by light mosaics (data not shown). For each of the ICs, the CP region was amplified by Phusion PCR (Thermo Scientific) (Primers 1+2, Table 1) and directly sequenced. Retention of the desired mutations was confirmed in progeny of the two mutant ICs, however secondary mutations in the CP region were also observed (Figure 1). The progenies of the parental IC and of the A-T78 mutant were found to contain an additional V-A230 mutation, while the A-T114 mutant progeny contained an additional E-K236 mutation.

Homogenates from the sequenced primary *N. benthamiana* infections were used to inoculate wild-type and *Rx*-expressing *N. benthamiana*, *S. lycopersicum* (cv. Microtom) and *N. tabacum* (cv. Samsun) (Bendahmane *et al*., 1999; Candresse *et al*., 2010). Triplicate plants of each host type were inoculated with sap representing each IC. Plants were grown as outlined previously. A phenotypic analysis and evaluation of systemic viral accumulation was carried out at 21 days post inoculation (dpi). ELISA readily detected the wild-type EU IC in all wild-type hosts, indicating full capacity for systemic movement and accumulation (Figure 2). The systemic infection phenotypes were characterised by light mosaics in *N. benthamiana* (Figure 3A), but by asymptomatic infection in *S. lycopersicum* (Figure 3B) and *N. tabacum* (data not shown). In contrast to the wild-type plants, background ELISA values were observed and a general absence of symptoms on upper non-inoculated leaves for the *Rx* hosts, indicating an *Rx*-specific inhibition of viral systemic infection (Figure 2; B-C). However, necrotic local lesions were observed on the inoculated leaves of *Rx*-expressing *N. benthamiana* (Figure 3C; panel A). Necrosis around the site of inoculation was also observed in *S. lycopersicum* (Figure 3D) but not in *N. tabacum* (data not shown).

RNA was extracted from systemically infected leaves of the wild-type plants using an RNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacture's protocol. The CP region was amplified as described above and cloned into pJET1.2 (Thermo Scientific). At least two clones were sequenced for each sample. As it is widely known the potexviral CP is the sole elicitor of the *Rx*-based resistance response (Bendahmane *et al*., 1995, 1999; Goulden *et al*., 1993), only the CP regions were analysed in this work. In all three hosts,

the V-A230 mutation previously observed in the inoculum source was retained in the sequenced progenies (Table 2).

Similar to the wild-type EU IC parent, mutant A-T78 was able to systemically infect all wild-type host species, as indicated by ELISA values comparable to those observed in the wild-type infections (Figure 2). Again, light mosaics were observed in *N. benthamiana* (Figure 3A) but asymptomatic infection in both *S. lycopersicum* (Figure 3B) and *N. tabacum* (data not shown). High ELISA values were observed in the non-inoculated tissues of all *Rx*-expressing *N. benthamiana* and *S. lycopersicum*, indicating a breakdown of *Rx*-resistance in these hosts (Figure 2; A-B). Infection phenotypes were characterised by vascular necrosis in the upper parts of the plant in *N. benthamiana* (Figure 3A and 3E), and by trailing necrosis over the entire plant in *S. lycopersicum*, with the plant showing a very stunted phenotype (Figure 3B). A phenotype of spreading necrosis was also observed in the inoculated leaves of *N. benthamiana* (Figure 3; panel B). In contrast to the situation in *Rx*-expressing *N. benthamiana* and *S. lycopersicum*, no symptoms or systemic viral accumulation could be detected in the *Rx*-expressing *N. tabacum plants*, indicating that mutant A-T78 could not evade the action of *Rx* in this host.

RNA was extracted from systemically infected leaves of both wild-type and *Rx*-expressing plants where infection had established (one plant representing each infection event), and the CP regions of the viral progenies sequenced as described above; the results are given in Table 2. The introduced A-T78 mutation and the V-A230 secondary mutation previously detected in the inoculum were retained in all progenies sequenced. However, in a third of progeny clones obtained from the systemic leaves of Rx expressing *N. benthamiana* the A-T78 mutations was lost and instead a D-E3 mutation was observed.

Similar to the A-T78 mutant, the A-T114 mutant possessed full systemic accumulation capacity in all wild-type hosts, indicated by positive ELISA values comparable to those of the parental isolate (Figure 2). The infection phenotypes were also similar to those observed for the wild-type EU IC (Figure 3A and 3B). The *Rx* resistance-breaking capability of this mutant was also found to differ between the three tested *Rx*-expressing hosts. Systemic accumulation levels similar to those in the wild-type host were only observed in *S. lycopersicum,* indicating *Rx*-breaking in this host (Figure 2; B), and the plants showed trailing necrosis phenotype (Figure 3B). However, no symptoms in non-inoculated tissues and no systemic accumulation could be observed for this mutant in *N. benthamiana* or *N. tabacum Rx*-expressers (Figure 2; A and C). A local necrotic response was observed in the inoculated leaves of *Rx*-expressing *N. benthamiana* (Figure 3; panel C), characterised by circular necrotic lesions within a background of complete leaf necrosis. Sequencing of CP regions was conducted for all progenies as described above; the results are given in Table 2. The E-K236 secondary mutation that had been identified in the inoculum was lost from all progenies. Instead, the same V-A230 mutation present in the inoculum source, progenies of the wild-type parent and that of the A-T78 mutant, was observed. In tomato, the introduced A-T114 mutation was retained in all progenies irrespective of the *Rx*-status of the plants, but was accompanied by an A-D100 secondary mutation in 75% of progeny clones obtained from *Rx*-expressing plants. In wild-type *N. benthamiana*, the A-T114 mutation was retained in all instances. On the contrary, it was absent in all progeny clones obtained from *N. tabacum* and instead a secondary D-E3 mutation was observed.

This work describes the analysis of the impact of the introduction of two point mutations on the infection phenotype in wild-type and *Rx*-expressing plants of three host species.

These mutations in the *Rx* minimal elicitor region of an *Rx*-sensitive PepMV IC of the EU genotype had been selected because they were expected to confer *Rx*-breaking properties (Candresse *et al*., 2010). The wild-type EU IC possessed full capacity for systemic movement and accumulation in all three wild-type hosts tested, but as expected from previous reports (Baures *et al*., 2008, Candresse *et al*., 2010), it was efficiently and specifically restricted in all *Rx*-expressing hosts, further confirming that PepMV is recognised by the *Rx*-sensing mechanism. It is interesting to note that localised necrotic responses were observed both at and around the site of inoculation for *N. benthamiana* and *S. lycopersicum*, while no such reaction was observed in *N. tabacum,* possibly as a consequence of *Rx* functioning more efficiently in this host. Previous work had shown *Rx* to confer a complete ER phenotype when confronted with a range of different avirulent potexviruses, including PepMV (Baurès *et al*., 2008; Candresse *et al*., 2010). One possibility for this discrepancy is that work carried out by Candresse *et al*. (2010) concerned the CH2 genotype of PepMV, while the IC used in the present investgation was of the EU strain.

A secondary mutation, V-A230, was observed in all wild-type EU IC progeny as well as in almost all progenies derived from the two mutants; the sole exception of which is the first progeny obtained in wild-type *N. benthamiana* for mutant A-T114. This mutation was observed irrespective of the *Rx*-status of the host species, suggesting that its highly reproducible accumulation likely reflects the reversion of a detrimental mutation present in the parental IC. In keeping with this interpretation, the alanine at position 230 is highly conserved among PepMV isolates and only absent in 3 out of 82 PepMV CP sequences present in Genbank, all three deriving from the EU IC used in the present experiments. On the other hand, the E-K236 mutation observed in the A-T114 inoculum, but lost upon

further propagation, is likely the result of unselected genetic drift. The same could be true for the D-E3 mutation observed in the progeny of the same mutant upon propagation in wild-type tobacco, but this remains to be conclusively demonstrated.

Mutation A-T78 was sufficient to confer *Rx*-breaking properties in both *N. benthamiana* and *S. lycopersium*, but not *N. tabacum*, without a need for any additional secondary mutation in the CP. This result is in line with previous work where the A-T78 mutation was identified alone in the CP region of *Rx* resistance-breaking variants of PepMV in tomato (Candresse *et al*., 2010). In contrast to the A-T78 mutant, A-T114 was only able to overcome *Rx* in *S. lycopersium* and not in *N. benthamiana* or *N. tabacum*. The role of mutation A-T114 in conferring *Rx*-breaking activity in tomato is less clear-cut than for A-T78 since a secondary A-D100 mutation was also observed in the majority of clones sequenced.

It would appear that mutation A-T114 confers *Rx*-breaking activity in *S. lycopersicum* but requires a second compensatory mutation such as the A-D100 reported here or the A-V71 previously observed together with A-T114 in spontaneous *Rx*-breaking mutants (Candresse *et al*., 2010). In all cases, *Rx*-breaking in *S. lycopersicum* was accompanied by a spreading necrosis phenotype (Candresse *et al*., 2010; present work) which is *Rx*-mediated as it is not observed in wild-type tomato. The secondary A-D100 mutation was observed in the majority of sequenced clones from *Rx*-tomato yet was absent from all sequences obtained from wild-type tomato, indicating its likely compensatory role. Interestingly this same A-D100 mutation has been observed in the CP region of two PepMV resistance-breaking variants in *S. lycopersicum* (Candresse *et al*., 2010), alongside Q-R125 in one variant, and with both A-T78 and Q-R125 in another.

The results of this investigation show *Rx* to posses a high level of recognition in tobacco as no resistance-breaking is observed and systemic movement of the virus is halted. In *N. benthamiana Rx*-recognition is intermediate. A114T is recognised (local lesions) but not localised, while A78T is not recognised and displays complete systemic movement capability. In *S. lycopersicum*, recognition is weakest and both mutants, while still recognised, evade localisation and overcome the resistance. Evidence for intermediate elicitor recognition phenotypes observed in CP-*Rx* based systems shows the intensity of the response may vary, clearly based on the strength of protein-protein interactions (Baurès *et al*., 2008; Sturbois *et al*., 2012). Indeed, the findings of this investigation nicely parallel those of Sturbois *et al*., (2012), whereby different tomato mutants were found to possess different interaction phenotypes when confronted with a mutant PVX isolate of intermediate *Rx*-elicitor activity. Work by Harris *et al*.**,** concerning the artificial evolution of *Rx*, found that extending the range of *Rx*-recognition to include *Poplar mosaic virus* could come with a cost of systemic trailing necrosis (Harris *et al*., 2013). The *Rx* resistance response was demonstrated to consist of separate recognition and activation phases, with PopMV being recognised but a delayed activation of Rx resulted in an HR insufficient to prevent viral spread. The necrotic symptoms displayed by PepMV mutants in *Rx*-expressing hosts in this work show the mutants are also recognised but not localised, but instead this is due to host-specific differences in the downstream signaling capabilities of Rx homologs between heterologous hosts.

Another point to consider may be host-dependent fitness penalties associated with each mutation. However, fitness penalties associated with the debilitating T121K mutation in PVX mutants are seen in non-*Rx* hosts (Goulden *et al*., 1993), therefore the equal levels of

fitness displayed between the mutants in the WT hosts in this work makes this mechanism unlikely.

In conclusion, the results of this investigation support the guard hypothesis of *R* gene functionality (Dangl and Jones, 2001), which implies the existence of host adapters that may contribute to resistance efficiency and durability. Understanding the mechanisms underlying the increased durability of *Rx*-based resistance in the *N. tabacum* host may be integral if *Rx* gene is to provide a suitable form of resistance against PepMV in tomato. This is a much more complex system than previously thought; the cellular environment in which *Rx* is expressed is integral in its functionality.

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Figure 1: Consensus sequences of the PepMV CP regions obtained from systemic leaves of *N. benthamiana* after inoculation with *in vitro* generated RNA representing each IC; EU, A-T78 and A-T114. Grey crosses indicate intended mutations and white stars indicate secondary mutations.

Figure 2: DAS-ELISA data displaying systemic viral titres 21 dpi of PepMV ICs; A-T78, A-T114 and unmutated EU, in *Rx*-expressing (Rx) and WT solanaceous hosts. (A) *N. benthamiana*, (B) *S. lycopersicum* Cv. Microtom and (C) *N. tabacum* Cv. Samsun.

Figure 3A: Representative phenotypes displayed in wild-type and *Rx*-expressing *N. benthamiana* by the wild-type EU IC and CP point mutation ICs A-T78 and A-T114. All ICs presented mild phenotypes in the wild-type hosts. Both EU and A-T114 were asymptomatic in the *Rx* host, while A-T78 presented vascular necrosis in the upper parts of the plant. Symptoms viewed 21 dpi.

Figure 3B: Representative phenotypes displayed in wild-type and *Rx*-expressing (Rx) *S. lycopersicum* by ICs EU, A-T78 and A-T114. All ICs displayed asymptomatic phenotypes in the wild-type hosts. In the *Rx*-expressing hosts the EU IC caused basal necrosis around the site of inoculation, while both A-T78 and A-T114 displayed trailing necrosis over the plant surface. Symptoms viewed 21 dpi.

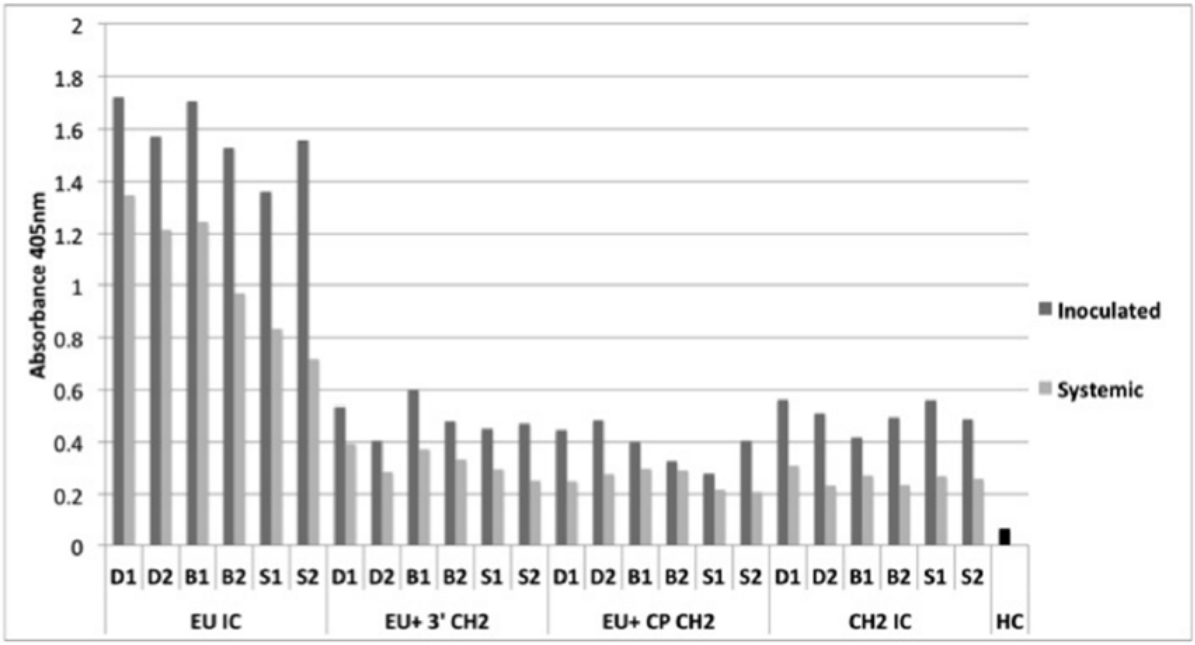
Figure 3C: Responses observed in the inoculated leaves of *Rx* expressing *N. benthamiana*, challenged with (A) EU IC, (B) mutant A-T78 and (C) mutant A-T114. Symptoms viewed 21 dpi.

Figure 3D: Close up of basal necrosis around the site of infection in *Rx*-expressing *S. lycopersicum* challenged with the WT EU IC. Symptoms viewed 21 dpi.

Figure 3E: Close up of vascular necrosis in *Rx*-expressing *N. benthamiana* challenged with mutant A-T78. Symptoms viewed 21 dpi.

Table 1: Primers and annealing temperatures used in this investigation.

Table 2: Mutational composition of clones sequenced from systemic infections generated in both *Rx* expressing and WT hosts from challenge with both WT and mutant ICs. Intended mutations are indicated in italics, secondary mutations in black. Infection phenotypes are also given.


Absorbance 405nm
2
1.8
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0.2
0
D1 D2 B1 B2 S1 S2 D1 D2 B1 B2 S1 S2 D1 D2 B1 B2 S1 S2 D1 D2 B1 B2 S1 S2
EU IC
EU+ 3' CH2
EU+ CP CH2
CH2 IC
HC
Inoculated
Systemic

Figure 1A

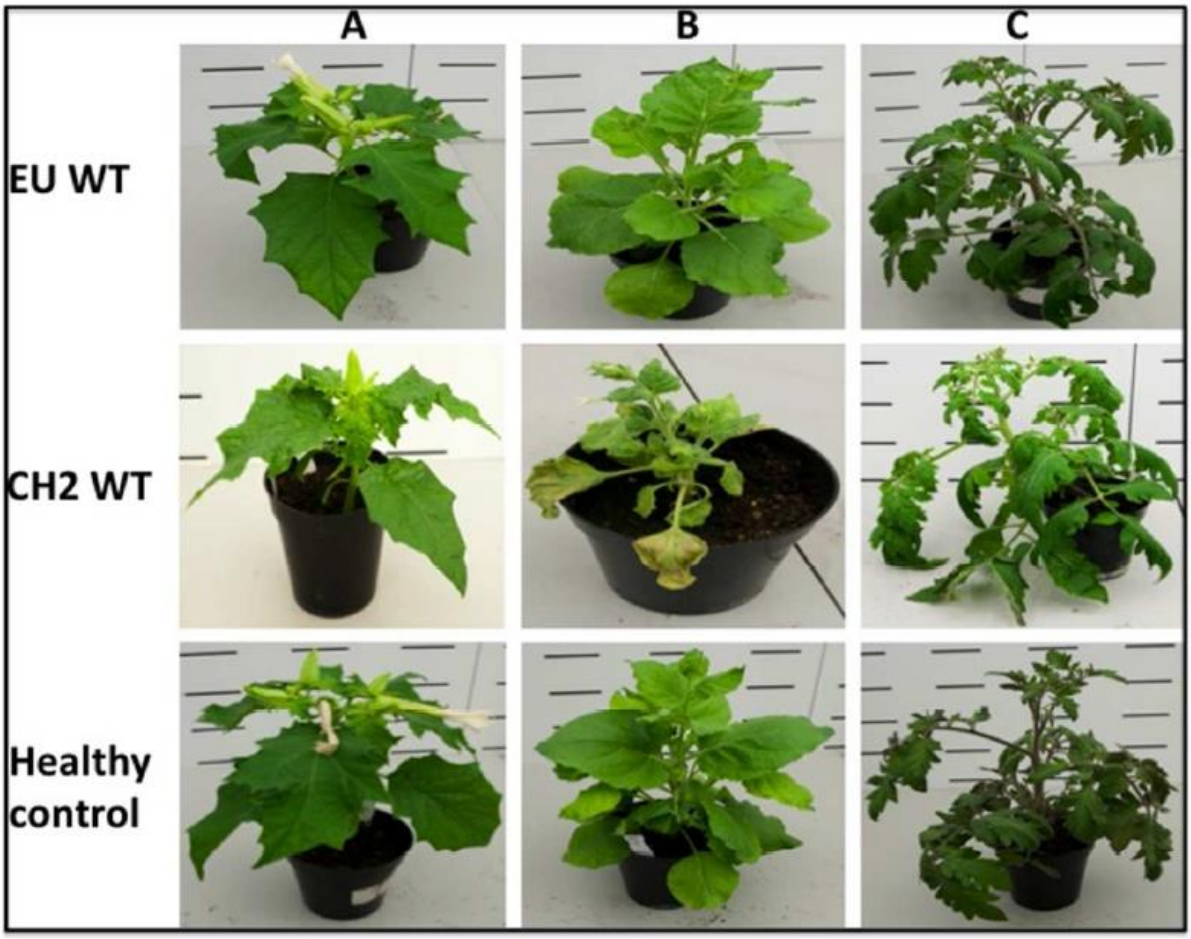

Figure 1A

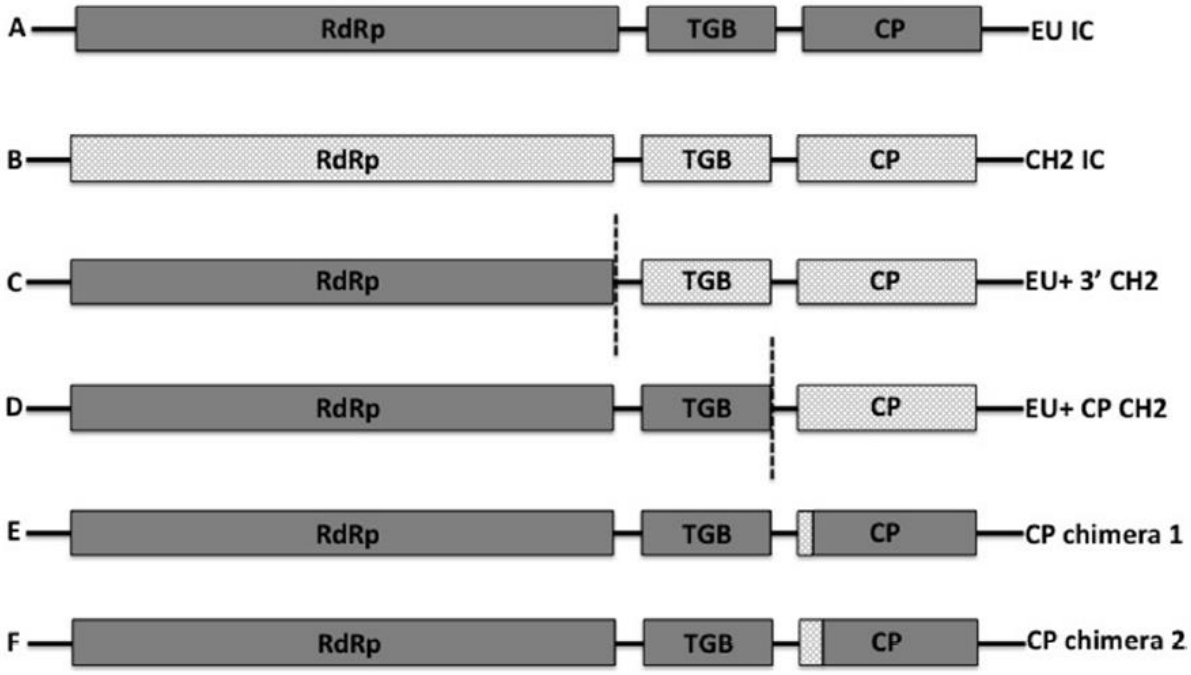
A
RdRp
TGB
CP
EU IC
B
RdRp
TGB
CP
CH2 IC
C
RdRp
TGB
CP
EU+ 3' CH2
D
RdRp
TGB
CP
EU+ CP CH2
E
RdRp
TGB
CP
CP chimera 1
F
RdRp
TGB
CP
CP chimera 2

Figure 2A

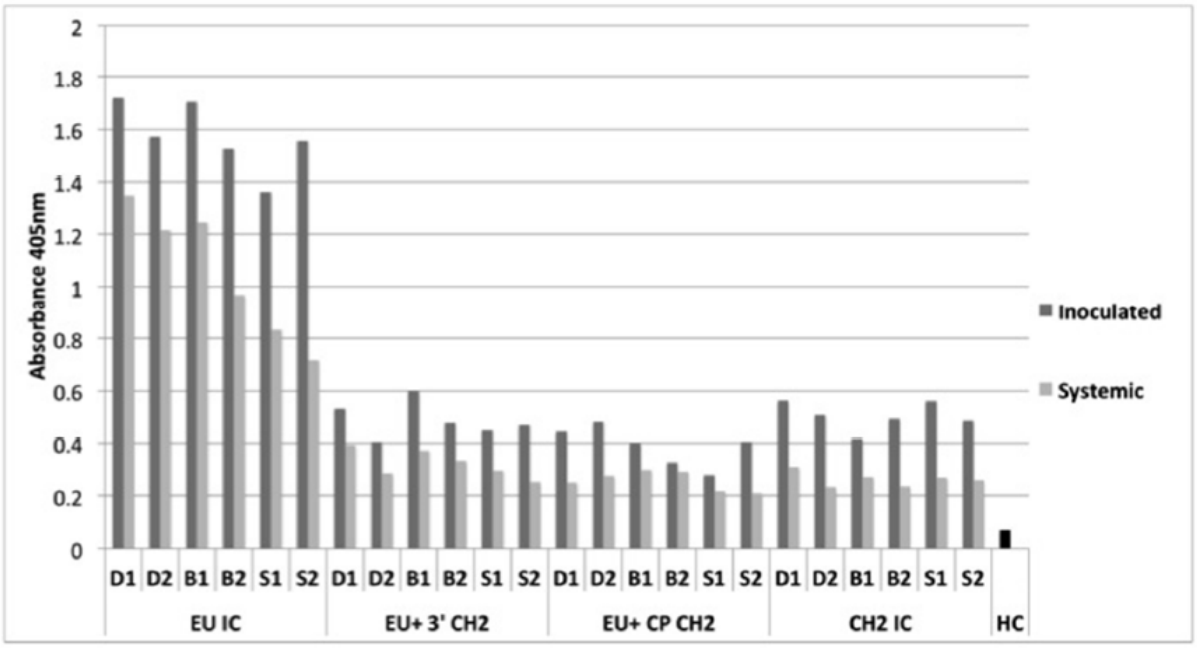
Absorbance 405nm
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
D1 D2 B1 B2 S1 S2 D1 D2 B1 B2 S1 S2 D1 D2 B1 B2 S1 S2 D1 D2 B1 B2 S1 S2
EU IC
EU+ 3' CH2
EU+ CP CH2
CH2 IC
HC
Inoculated
Systemic

Figure 2B

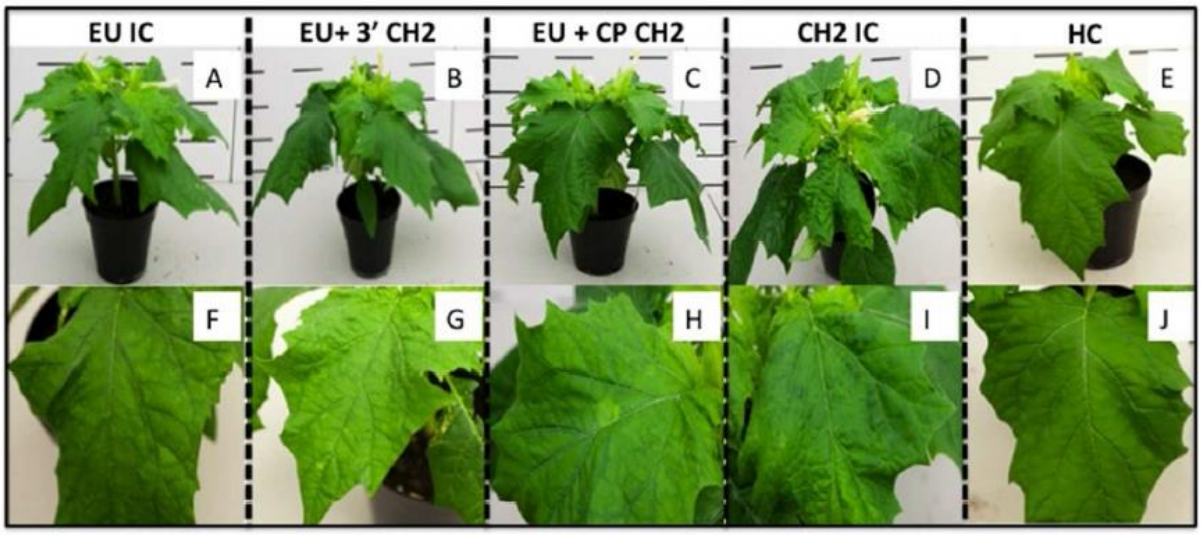

Figure 2C

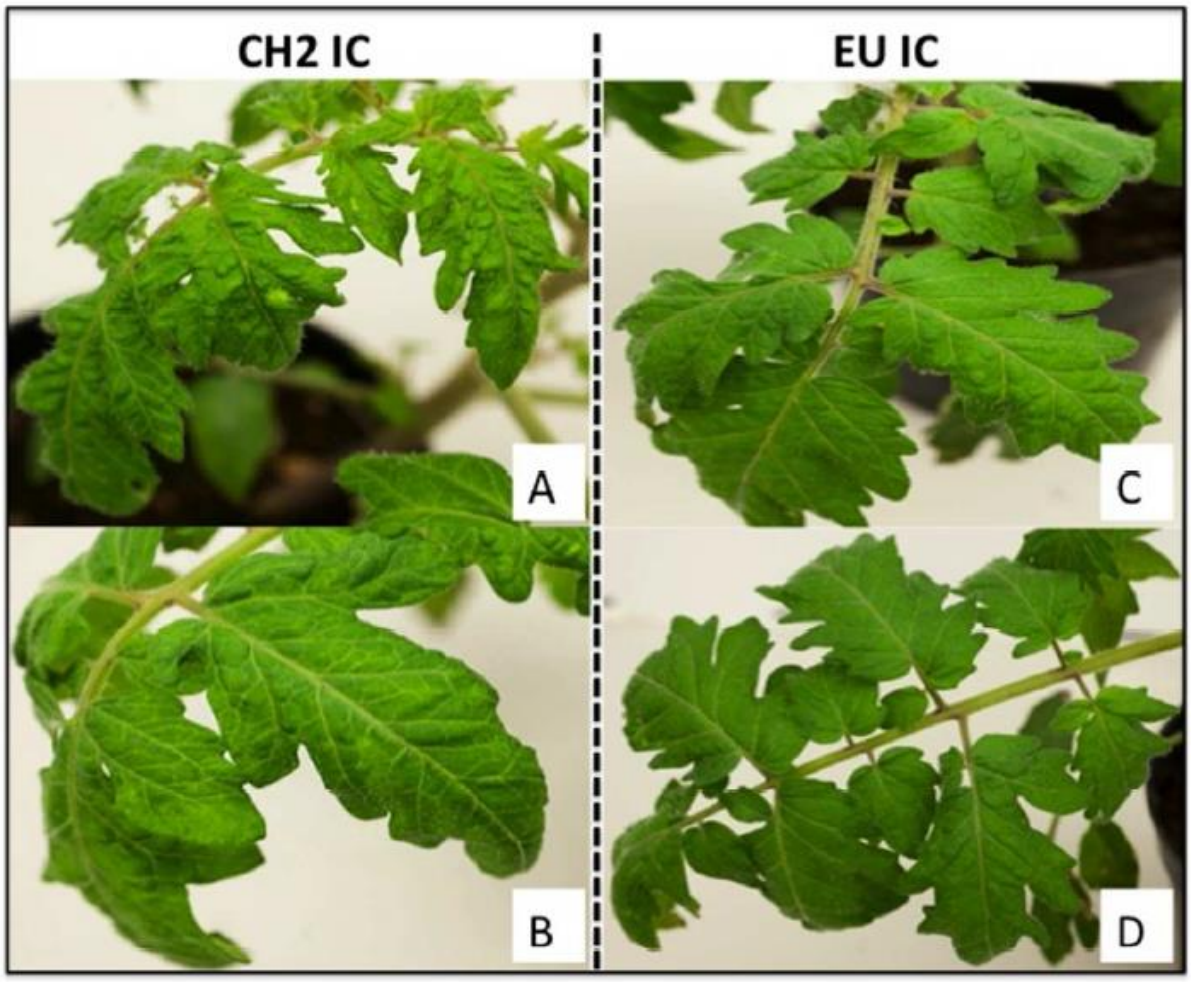

Figure 2D

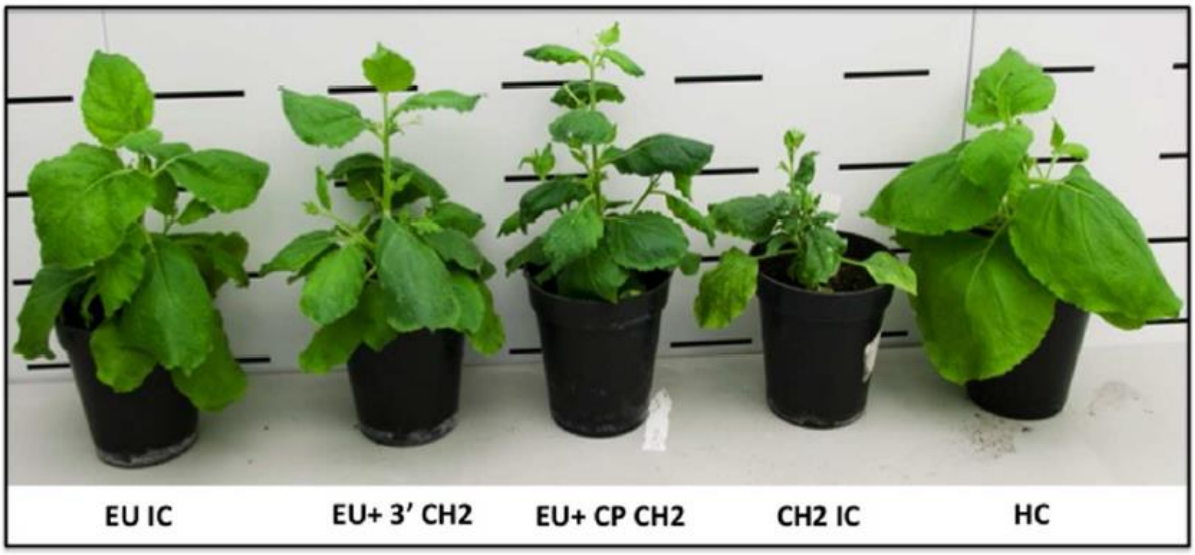

Figure 2E


2
1
EU IC 1 mpdttpvaatssapptakdagakapsdfsnpntapslsdlkkvkyvstvtsvatpaeiealgkiftamglaanetgpamwdlarayadvq
CH2 CP 1 menqptasnpsdvpptaaqagaqspadfsnpntapslsdlkkikyvstvtsvatpaeiealgkiftamglaanetgpamwdlarayadvq
EU IC 271 ssksaqligatpsnpalsrralaaqfdrinitprqfcmyfakvvwnilldsnippanwaklgyqedtkfaafdffdgvtnpaslqpadgl
CH2 CP 271 ssksaqligatpsnpalsrralaaqfdrinitprqfcmyfakivwnilldsnvppanwaklgyqedtkfaafdffdgvtnpaslqpadgl
EU IC 541 irqpnekelaahsvakygalarqkistgnyittlgevtrghmggantmyvidappel*
CH2 CP 541 irqpnekelaahsvakygalarqkistgnyittlgevtrghmggantmyaidappel*

Figure 3A

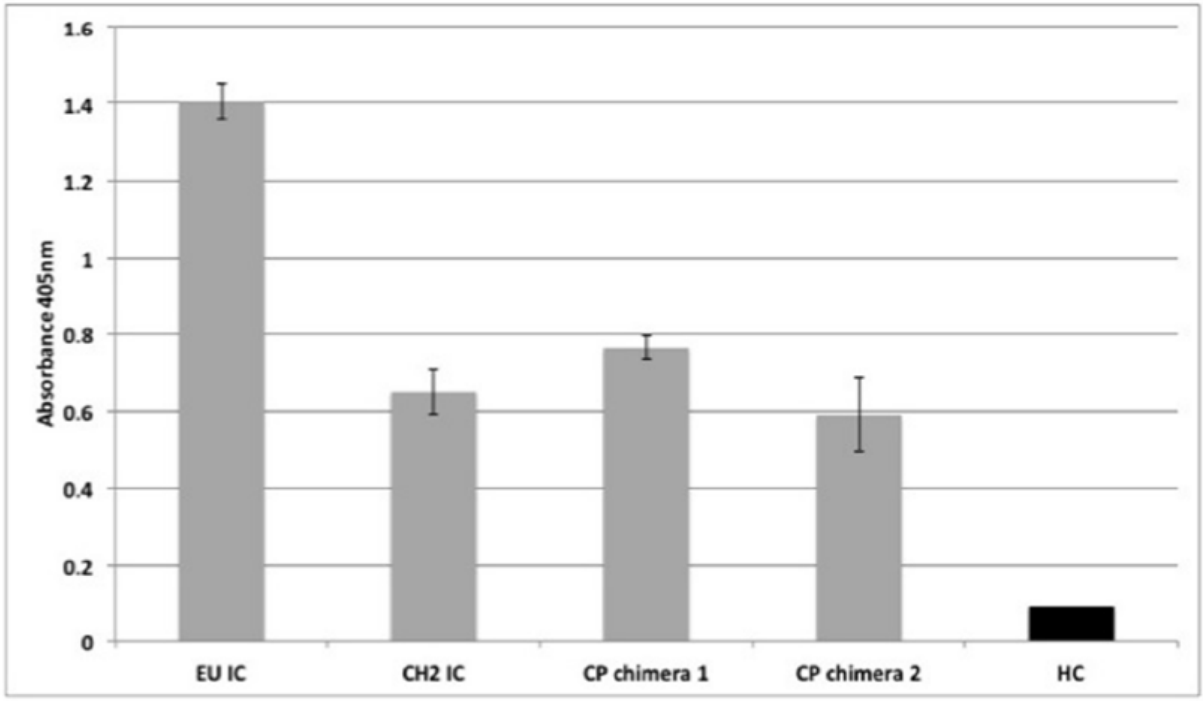
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbance 405nm
EU IC
CH2 IC
CP chimera 1
CP chimera 2
HC

Figure 3B

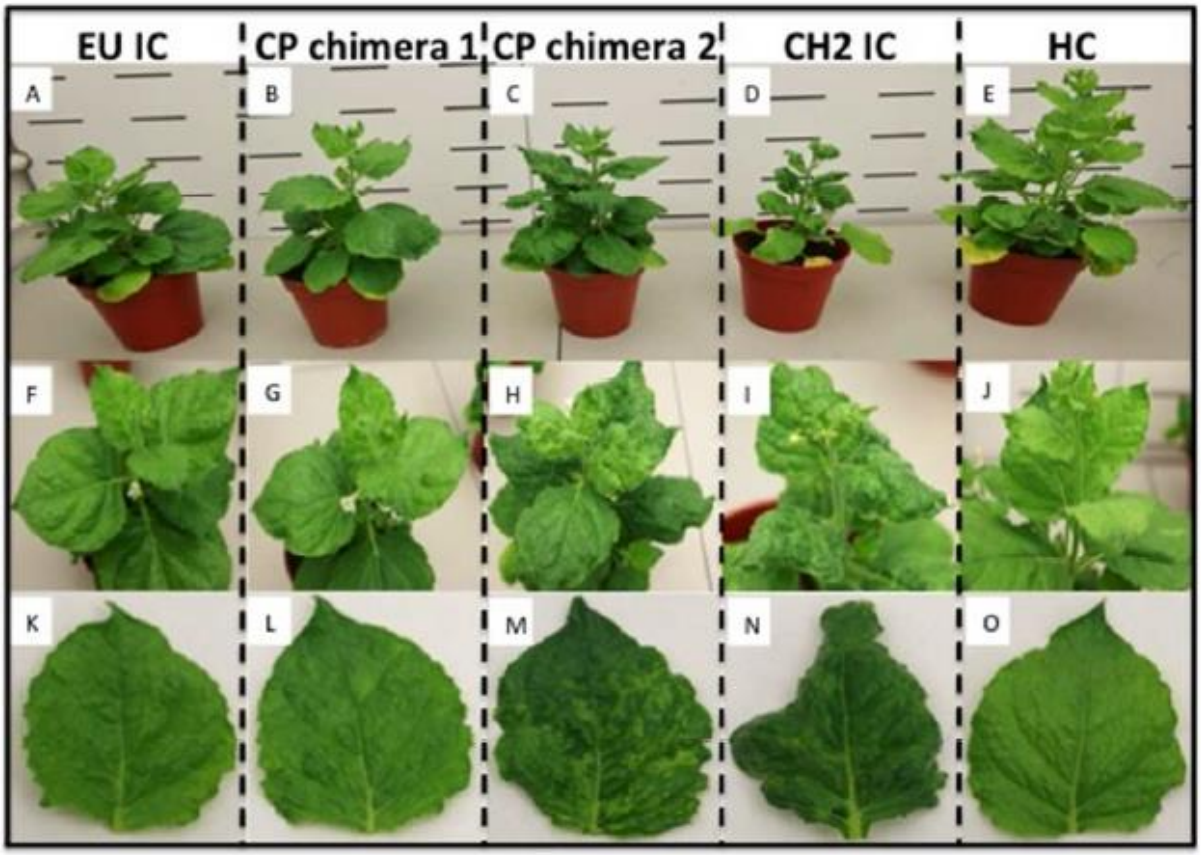

Figure 3C

Primer number	Primer sequence (5′- 3′)	Annealing temperature (°C)
1	GACATGAAGCATTCATACCAAATGGG	58
2	GAGCGGCCGCCAGTGTGATGGATATCTGCAAATTACTAGTATTTAGGTGACACTATAGAAAACAAAATAAATAAATAAATATA	61.5
3	ATGGTGGAACAAATAGGCTTCAATGTAACC	61.5
4	TGATTGGGTGAACAAATTGTGTAAGCTACC	61.5
5	TAACCCCTCAATGTGTTGCTTTTGGAGGGC	61.5
6	AAACATTCATACCAAATGGGTGATGAGCTG	61.5
7	CTCACTATAGGGAATATTAAGCTTGGTACCAATTGGTACCACGCGTTTTTTTTTTTTTTTTTTTTTTTTT	61.5
8	GAGCGGCCGCCAGTGTGATGGATATCTGCAAATTACTAGTATTTAGGTGACACTATAGAAAACAAAACATAACACATAATATC	60
9	GTAATGCCTGGGATTTGCCAGAACCTCCGC	60
10	TATGCCATCCCAATAAGCCACAGGAGGGC	60
11	GTCACCCATTTGGTATGAATGCTTCATGTC	60
12	GGGGATTACGCTGAATTTTGCGGTTGGAC	60
13	GGT AAA GTT TGA CCC CTT TTG AAT TGG GGA GTT ACA CAA CGA AAA CCT CCC GAG AAA AGT GGT TC	58
14	TGA TGA GCT GCA CAA TTA CTT AAC ACC AGA TGA AGC TGA ACA ACA CTT CCT TGC TGT TC	58
15	AGAGTCCCACATTACACTTCCTTC	58
16	TGG GTT AGA AGC TGT AGG TTG GTT TTC CAT GAT TGT TTA TTG AAG TTG AT	58
17	ATG GAA AAC CAA CCT ACA GCT TCT AAC CCA TCA AGT GCA CCA CCC ACA GCC	58
18	ATG GAA AAC CAA CCT ACA GCT TC	58
19	GGC TGG GCT CTG GGC ACC AGC	58
20	CTG CTG CTC AAG CTG GTG CCC AGA GCC CAG CCG ACT TCT CAA ATC CCA ATA CAG	58